

	PCR 20 µL reaction (units : uL)	Final Concentration				Concentration:	Water volume [µl]:
5X Phusion HF Buffer	4	1X		DNA:	CYC_1	12,4	11,4
10 mM dNTPs	0,4	200 µM			CYC_2	11	10
10 µM Forward Primer	1	0.5 µM			CYC_3	10,3	9,3
10 µM Reverse Primer	1	0.5 µM					
G-block (0.1ng/µL)	1						
DMSO (optional)	0,6	3 %		Primers:	f_Gbs_CYC		
Phusion DNA Polymerase	0,2	1.0 units/50 µl PCR			r_Gbs_CYC		
Nuclease-free Water	11,8						
Total volume	20						
Number of reactions (including controls):							
WORK ON ICE	CYC						
number of reactions:	4						
Mastermix	volume in uL						
5X Phusion HF Buffer	16	x					
10 mM dNTPs	1,6	x					
10 µM Forward primer	4	f_Gbs_CYC					
10 µM Reverse primer	4	r_Gbs_CYC		Lanes	Product		
DMSO (optional)	2,4	x		-	Generuler 1kb ladder		
Phusion DNA Polymerase (ADDED LAST)	0,8				1	25,5	
Nuclease-free Water	47,2	x			2	29,5	
	76				3	c3_0	
Volume of master mix in each tube:	19 uL				4	c3_3	
					5	pCYC-yeGFP circular	
					6	pCYC_yeGFP lin 1	
PCR programming:					7	pCYC_yeGFP lin 2	
Initial Denaturation	98°C	30 seconds			8	CYC_1 gbs	
	98°C	7 seconds					
	58°C	20 seconds					
35 Cycles	72°C	30 seconds			9	CYC_2 gbs	
Final Extension	72°C	10 minutes			10	CYC_3 gbs	
Hold	4°C				11	negative control	